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Adaptive Ecology of *Lotus corniculatus* L. Genotypes: I. Plant Morphology and RAPD Marker Characterizations

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ABSTRACT

Birdsfoot trefoil (*Lotus corniculatus* L.) is a highly variable and widely distributed Old-World perennial forage legume found in wild and naturalized populations throughout temperate regions of Europe, Asia Minor, North Africa, North and South America, Australia, and New Zealand. Understanding the relationships among birdsfoot trefoil morphologic, ecogeographic, and genetic characteristics may provide insights for better utilizing exotic germplasm. The objectives of this research were to (i) compare morphologic and random amplified polymorphic DNA (RAPD) classifications of 28 exotic and ecologically diverse genotypes from the USDA National Plant Germplasm System (NPGS) birdsfoot trefoil collection, and (ii) determine the relationships between genotype classifications and collecting-site ecogeographic features. Eighteen morphologic characteristics, 130 RAPD bands, and eight collecting-site ecogeographic characteristics were used to classify the genotypes. The relatedness of genetic, morphologic, ecologic, and geographic distances among the genotypes was measured using the product moment correlation. Genotype morphology was related to collecting-site distances from one another and ecologic similarity. Genetic relatedness was also associated with collecting-site ecology, and specific morphologic characteristics were associated with different ecogeographic features. The similarity between the genetic and ecologic classifications suggested that genotypes adapted to similar habitats, even if geographically distant, have acquired similar phenotypes. Since RAPD descriptors were associated with the ecologic similarity of genotype collecting sites but not with their geographic closeness, classifications of birdsfoot trefoil should rely on both ecogeographic and morphologic characteristics of accessions.

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THE GENUS *Lotus* is a large polymorphic and widely distributed group that comprises approximately 200 annual and perennial species (Grant, 1965). Birdsfoot trefoil is generally reported to have $2n = 4x = 24$ somatic chromosomes, but diploid populations have also been reported (Grant and Small, 1996). Birdsfoot trefoil is the most agriculturally important species of the genus, has a high nutritive value, and is non-bloating when grazed directly by livestock (Seaney and Henson, 1970; Beuselinck and Grant, 1995).

Birdsfoot trefoil is native to Europe and western Asia, but is also widely naturalized throughout temperate regions of South and North America, Australia, and New Zealand. Much of birdsfoot trefoil's wide range of adaptation has resulted in its genetic diversity. The range of phenotypes found in birdsfoot trefoil is believed to have developed as a result of adaptation to the environments in which it is found and through continual intraspecific hybridization (Chrtková-Zertová, 1973). Birdsfoot trefoil is tolerant of acid, infertile, and poorly drained soils where other popular forage legumes do not thrive. The greatest genetic diversity for birdsfoot trefoil occurs in the Mediterranean Basin (Grant, 1991).

The role of germplasm collections in the improvement of cultivated plants has been long recognized (Frankel and Hawkes, 1975; Hawkes, 1983; Holden and Williams, 1984). Germplasm collections can contain genes for resistance to pests, diseases, and abiotic stresses, and help to ensure that potentially useful genetic variation is preserved for future needs (Astley, 1987). Ex situ collections of exotic germplasm collected from populations

Abbreviations: GRIN, Genetic Resources Information Network; NPGS, National Plant Germplasm System; RAPD, random amplified polymorphic DNA.

growing in the wild have contributed to the development of improved cultivars and are genetic repositories for future breeding efforts of agriculturally important plants.

Birdsfoot trefoil has been shown to be a highly variable species. Genetic diversity has been shown for cyanogenic reaction (Blaise et al., 1991), seed globulin polypeptides (Steiner and Poklemba, 1994), random amplified polymorphic DNA (RAPD) (Campos et al., 1994), and rDNA internal transcribed spacer sequence polymorphisms (Steiner, 1999a). Variability also exists for specific agronomic traits, including herbage regrowth and quality, flowering habit, insect resistance (Chrtková-Zertová, 1967, 1973; Seaney and Henson, 1970; Duke, 1981; McGraw et al., 1989), and reproductive compatibility (Garcia de los Santos et al., 2001). However, even with the great range of genetic diversity available for genetic improvements, examination of birdsfoot trefoil cultivar pedigrees and biochemical marker profiles has shown that a limited genetic base has been used for cultivar development (Steiner and Poklemba, 1994). Such genetic bottlenecks are a common problem with many forage species where plant breeders have used previously developed cultivars as the genetic stock to develop new cultivars (Rumbaugh, 1991). To broaden its genetic base and efficiently use more diverse genetic resources, more must be understood about the existing genetic relationships within and among birdsfoot trefoil and its closely allied relatives.

Understanding the genetic diversity within germplasm collections facilitates use of genetic diversity and has been cited as a reason for characterizing germplasm collections (Strauss et al., 1988; Beuselinck and Steiner, 1992). Since birdsfoot trefoil is morphologically variable and easily confused with similar species, it is difficult to delimit hybrid types among botanic varieties within its range of phenotypic variation (Chrtková-Zertová, 1973). A combination of different kinds of plant descriptors may be necessary to accurately distinguish accessions within germplasm collections. However, only partial information about birdsfoot trefoil germplasm resources has been assembled from different research efforts.

To identify genetic materials that may contain useful traits for germplasm enhancement, a systematic evaluation of genetic diversity is needed to understand relationships among accessions and their corresponding collecting-site environments (Steiner and Greene, 1996; Steiner, 1999a). As more is understood about genotypic and phenotypic plant-descriptor relationships and how these are related to collecting-site ecologic features and distributions, it may be possible to develop insights into ecologic adaptations useful for crop improvement (Rick, 1973), predict where unique accessions may be found in the wild (Vavilov, 1992), and identify sources of materials that contain desirable traits (Steiner and Poklemba, 1994; von Bothmer and Seberg, 1995; Steiner and Greene, 1996). The objectives of this research were to (i) characterize and compare morphologic and RAPD marker classifications of 28 ecologically diverse genotypes from the NPGS birdsfoot trefoil collection, and (ii) determine the relationship of RAPD and morphologic descriptors with ecogeographic characteristics of genotype collecting sites.

MATERIALS AND METHODS

Genetic Materials

Original seeds of most accessions (Table 1) were obtained from the USDA-ARS Plant Introduction Station at Pullman, WA. Based on accession descriptions, all were presumed to be wild or landrace cultivars and thus referred to as exotic. The seeds were scarified using liquid nitrogen, germinated at 20°C in plastic boxes on blue blotter paper, and inoculated with an appropriate *Rhizobium* strain; the germinated seedlings were transplanted to greenhouse flats. Approximately 15 plants of each accession were grown in the greenhouse under 16/8 h (light/dark) conditions at approximately 20°C. Plants were fertilized, watered, and treated for insect and disease pests as needed. All of the genotypes (except PI 260268 from Ethiopia that required vernalization in a growth chamber at 2°C for 4 wk) flowered at ambient temperature under 16/8 h (light/dark) photoperiod conditions in the greenhouse during a 3-yr period. The plants were periodically rearranged on the greenhouse benches.

All plants from each accession were visually examined for morphologic uniformity regarding growth habit, leaf shape and color, amount of pubescence, and flowering. Based on the general morphologic uniformity among all plants within each accession, one representative genotype was chosen at random from each 15-plant accession population and transplanted into a 2.8-L pot filled with a commercial potting soil mix. All morphologic characterizations were based on these representative individuals from each accession. The approximately 14 remaining genotypes of each accession were maintained in 10-cm diam. pots for observation throughout the experiment.

To assess intra-accession genetic variation and validate the use of the single genotype to represent each accession population, five to six plants (including the representative genotype) each from a subset of eight ecologically and morphologically diverse accessions (Table 1: MOR, SWI, FRA-2, ETH, NOR-2, RUS-2, TUR, and NC-83, a commercial cultivar) were selected and analyzed for RAPD marker variation. An analysis of bulked leaf samples from the 15 genotypes of each of the eight accessions was also included in the RAPD analysis. The relative amount of intra- vs. inter-accession genotypic variation based on RAPD band genetic distance (see methods below) within the eight accessions tested was determined using analysis of variance and tested using the F statistic (Neter and Wasserman, 1974).

Collecting-Site Ecogeographic Descriptions

Twenty-eight exotic birdsfoot trefoil accessions from the USDA-NPGS birdsfoot trefoil collection were selected based on the geographic and ecologic diversity of the sites from which they were originally collected (Table 1). The latitude and longitude of the accession collecting sites were determined from original passport information found in the USDA Genetic Resources Information Network (GRIN) or estimated by retroclassification (Steiner and Greene, 1996) when actual collecting-site coordinates were not recorded. The environmental and ecological characterization data (Table 2) were acquired from the U.S. Environmental Protection Agency and National Oceanic and Atmospheric Administration (EPA/NOAA) Global Ecosystems Databases (Kineman and Ohrenschall, 1992, 1994). The 28 collecting sites were described by (i) ecoregions of the continents (Bailey, 1989); (ii) lowest

Table 1. Geographic origins of 28 exotic birdsfoot trefoil genotypes.

Entry	Identification	Country	City	Location	
				Latitude	Longitude
no.				°	
PI 31276	MOR	Morocco	Sefrou	33.63 N	04.87 W
PI 180171	CZE	Czech Republic	Tabor	49.25 N	14.41 E
PI 227512†	IRA-1	Iran	Komkun	29.00 N	53.00 E
PI 234670	FRA-1	France	Montombam	48.05 N	01.41 E
PI 234811	SWI	Switzerland	Chur	46.87 N	09.53 E
PI 235525†	FRA-2	France	Montepellier	43.36 N	03.53 E
PI 251143†	MAC	Macedonia	Skopje	41.59 N	21.26 E
PI 260268	ETH	Ethiopia	DireDawa	09.37 N	41.52 E
PI 260692†	ITA-1	Italy	Perugia	42.50 N	12.50 E
PI 267060	POL	Poland	Warsaw	52.15 N	21.00 E
PI 290717†	UK	United Kingdom	Reading	51.70 N	00.98 W
PI 93-94	GEO-2	Georgia	Kasbegi	41.43 N	44.49 E
PI 315082†	KAZ	Kasakstan	Alma-Ata	43.15 N	76.57 E
PI 315454	RUS-1	Russia	St. Petersburg	59.92 N	30.25 E
PI 319021	SPA	Spain	La Ercin	43.53 N	05.34 W
PI 319822	NOR-1	Norway	Oppland	61.10 N	09.40 E
PI 319823	NOR-2	Norway	Rosendal	59.59 N	06.01 E
PI 325369	RUS-2	Russia	Stavrop	45.02 N	45.59 E
PI 325379	UKR	Ukraine	Yalta	44.30 N	34.10 E
PI 369278	RUS-3	Russia	Novosibersk	55.02 N	82.55 E
PI 464682	TUR	Turkey	Akdagma	36.60 N	29.90 E
PI 384882†	IRA-2	Iran	Parvar	35.30 N	53.25 E
PI 419228	GRE-1	Greece	Nikitas	40.14 N	23.39 E
PI 419233	GRE-2	Greece	Kastaneai	41.38 N	26.28 E
PI 430546	RUS-4	Russia	Dedinovski	55.03 N	39.07 E
PI 93-21	GEO-1	Georgia	Khulo	41.41 N	42.18 E
PI 485601	ITA-2	Italy	Abbadia	45.23 N	11.41 E
PI 494653	ROM	Romania	Gheorghe	46.14 N	26.44 E

† Original collecting site seeds of this accession were not available.

(low) and highest (high) monthly temperature, annual average (average) temperature, monthly and annual accumulated precipitation (precipitation), monthly and annual accumulated snow depth (snow), monthly and annual percentage of sunshine hours (sunshine) (Leemans and Cramer, 1992); and (iii) modal elevation (elevation) (FNOC, 1992).

The ecologic distances (D_{eco}) among the collecting sites were determined by calculating the Euclidean distances from the effects of an aggregate of individual ecogeographic characteristics describing each collecting site. Included in the ecologic distance were lowest and highest monthly temperature, annual average temperature, monthly and annual accumulated precipitation, monthly and annual accumulated snow depth, and monthly and annual percentage of sunshine hours.

An estimate of geographic distances (D_{geog}) among all collecting sites was based on latitude and longitude coordinates using the equation developed by A. Afonin, Vavilov Research Institute, St. Petersburg, Russia (personal communication, 1996):

$$D_{geog} = \sqrt{\{(\text{Long}_a - \text{Long}_b) \times (\pi r/360) \times [\cos(\text{Lat}_a) + \cos(\text{Lat}_b)]\}^2 + \{(\pi r/180) \times [\cos(\text{Lat}_a) + \cos(\text{Lat}_b)]\}^2} \quad [1]$$

where Long_a and Long_b are the longitudes of collecting sites a and b being compared, respectively; cos (Lat_a) and cos (Lat_b) are the cosine of the latitudes of collecting sites a and b being compared, respectively; and r is the radius of the earth (6378 km).

Genotype Descriptions

Morphologic Characters

Eighteen morphologic characteristics that comprised 12 qualitative and 6 quantitative traits based on earlier descriptions by Chrtková-Zertová (1973) were used to assess the range of intraspecific morphologic variation among the genotypes (Table 3). Ten observations were measured per morphologic characteristic except for number of flowers per umbel, where 20 observations per genotype were used. Measurements were made on mature plants that had begun flowering.

A binary data matrix was constructed to describe the qualitative and quantitative morphologic characteristics (71 character states) and to determine the aggregate morphologic distances among genotypes. The pairwise morphologic distance (d_{morph}) among the 28 genotypes was calculated using PAUP 4 software for the Macintosh (Swofford, 1998) as described by Steiner (1999b) and using the representation:

$$d_{morph} = 1 - a\ b^{-1} \quad [2]$$

where a is the number of shared characters (character state present or absent), and b is the total number of character states scored.

Genetic Characters

Plant DNA was isolated by the method described by Steiner et al. (1995). Fresh leaves (≈3 cm^2) were collected and put into 1.1-mL tubes, each containing five to six 3.0-mm diam. glass beads, which were held in microtiter format racks, frozen in liquid nitrogen, lyophilized, and ground into a fine powder at room temperature by shaking. In addition, leaf samples were collected from each of the remaining 15 plants within the selected subset of accessions, bulked, and handled as above with modification for the larger sample size by using 2-mL tubes. Two replicates of 30 to 40 mg of ground leaf material from the samples were incubated, with occasional gentle mixing, in 1.0 mL of extraction buffer containing 100 m*M* Tris-HCl (pH 8.0), 1.4 *M* NaCl, 20 m*M* EDTA (ethylenedinitrilo tetraacetic acid), 20 g L^{-1} CTAB (hexadecyltrimethylammonium bromide), 10 g L^{-1} PVP-40, and 20 mL L^{-1} 2-mercaptoethanol at 60°C for 30 to 40 min. Chloroform (800 mL) was added

Table 2. Ecologic descriptions of collecting sites for 28 exotic birdsfoot trefoil genotypes.

	Ecoregion†			Ecological descriptors						
						Temperature				
Id.	Code	Domain	Division	Elevation	Precipitation	Average	Low	High	Snow	Sunshine
				m	mm		°C		cm	%
MOR	M262	Humid temperate	Mediterranean	1680	770	11	4	22	0	72
CZE	L222	Humid temperate	Hot continental	460	581	7	−3	17	35	37
IRA-1	M314	Dry	Tropical/Subtropical/Steppe	1980	298	15	5	25	0	68
FRA1	L242	Humid temperate	Marine	90	696	11	4	17	3	38
SWI	M243	Humid temperate	Marine/Mountain	1580	1075	−2	−11	7	124	39
FRA-2	L313	Dry	Tropical/subtropical/Steppe	30	146	19	10	29	0	70
MAC	M262	Humid temperate	Mediterranean/Mountain	910	496	5	−6	17	16	46
ETH	M412	Humid tropical	Savanna/Mountain	2130	685	17	13	19	0	67
ITA-1	L261	Humid temperate	Mediterranean	300	949	14	4	23	9	53
POL	L222	Humid temperate	Hot continental	90	484	8	−4	19	35	34
UK	L243	Humid temperate	Marine	90	645	10	4	17	0	32
GEO-2	M252	Humid temperate	Mediterranean/Mountain	800	1695	0	−11	11	76	43
KAZ	M341	Dry	Temperate Desert/Mountain	1520	480	−6	−23	9	99	53
RUS-1	L212	Humid temperate	Warm/Continental	100	466	4	−8	17	45	30
SPA	M243	Humid temperate	Marine/Mountain	700	909	8	3	15	0	43
NOR-1	M242	Humid temperate	Marine	910	515	1	−10	12	156	30
NOR-2	L244	Humid temperate	Marine	760	2532	1	−7	10	17	26
RUS-2	L343	Dry	Temperate desert	30	267	10	−6	26	6	42
UKR	M252	Humid temperate	Prairie	60	452	8	−2	20	1	54
RUS-3	L137	Polar	Subarctic	120	388	1	−18	19	195	42
TUR	M262	Humid temperate	Mediterranean/Mountains	1290	333	8	−7	20	39	55
IRA-2	M312	Dry	Tropical/Subtropical/Steppe	910	842	13	4	24	0	69
GRE-1	L261	Humid temperate	Mediterranean	460	513	15	4	25	6	58
GRE-2	L261	Humid temperate	Mediterranean	150	580	13	3	24	0	52
RUS-4	L212	Humid temperate	Warm continental	150	533	4	−13	18	69	36
GEO-1	M252	Humid temperate	Mediterranean/Mountains	900	637	3	−9	14	109	49
ITA-2	L243	Humid temperate	Marine	60	751	13	1	24	8	43
ROM	M252	Humid temperate	Prairie	910	525	6	−5	16	28	42

† From Bailey, 1989.

to each sample at equal volume, gently mixed, and centrifuged at 11 000 × *g* for 2 min. In a new tube, the upper aqueous phase was added to 750 mL of isopropanol, gently mixed, incubated at −20° C for at least 15 min, and then centrifuged at 11 000 × *g* for 10 min. The supernatant was discarded and the pellet washed in 1 mL of 700 mL L^{-1} ethanol, air-dried, and re-suspended in 125 mL of 10 m*M* Tris-HCl (pH 8.0) and 1 m*M* EDTA. The quantity, purity, and integrity of the genomic DNA was determined by electrophoresis with known amounts of lambda DNA in a 7 g L^{-1} Seakem agarose (FMC, Rockland, ME) gel in 1x Tris-acetate EDTA. The samples were diluted to approximately 1 ng mL^{-1} for use in the RAPD reactions.

Approximately 10 μL of the original extract, diluted 170-fold with 1690 μL of water, was used for the RAPD analyses. Three microliters of diluted extract was used in a 15-μL reaction mixture as a template for the RAPD reactions that contained 50 m*M* Tris-HCl (pH 9), 45 m*M* ammonium sulfate, 1.5 m*M* magnesium chloride, 100 m*M* dNTPs, 0.2 m*M* 10-mer primer (Operon, Alameda, CA), 1U Tfl DNA polymerase (Epicentre Technologies, Madison, WI), and overlaid with 50 μL of mineral oil. The reactions were run in an MJ Research (Watertown, MA) 96-well microtiter format thermocycler (PTC-100) using the temperature profile: 95°C for 2 min 30 s, 46°C for 40 s, 72°C for 1 min, 94°C for 40 s, 46°C for 40 s, 72°C for 1 min, 41 cycles of 40 s each at 94°C, and a final 9 min at 72°C. Random amplified polymorphic DNA products were separated by electrophoresis using 11 μL of the reaction mixture in a 17.5 g L^{-1} 3:1 NuSieve agarose gel (FMC, Rockland, ME) in 1x Tris-borate EDTA. The gels were run at a constant 90 mA, including a 100-bp DNA ladder standard (Gibco-BRL, Gaithersburg, MD) lane in each gel. Following electrophoresis, the bands were visualized by ethidium bromide staining and photographed under ultraviolet light with Polaroid 667 film (Polaroid, Cambridge, MA). Photographs

Table 3. Plant morphologic characters and their character states used to describe 28 exotic birdsfoot trefoil genotypes.

Plant character†	Plant character states
Whole plant	
Growth habit	(i) erect, (ii) ascending, and (iii) decumbent.
Underground shoots	(i) present, and (ii) not present.
Plants color	(i) glaucous, (ii) bright green, (iii) dark green, and (iv) brownish green.
Stems	
Stem firmness	(i) solid, and (ii) hollow (by transverse cuts).
Peduncle length	mm; measurements of well-developed secondary branches (three categories).
Leaves	
Leaflet form	(i) narrow oblanceolate, (ii) oblanceolate, (iii) obovate, (iv) round, and (v) obcordate.
Leaflet thickness	(i) thin, (ii) slightly fleshy, and (iii) fleshy.
Leaflet indumentum	(i) glabrous, (ii) ciliate, and (iii) hairy.
Central leaflet length	mm; central leaf, well developed branches (six categories).
Central leaflet width	mm; central leaf, well developed branches (three categories).
Flower	
Flowers per umbel	20 random undisturbed umbels (seven categories).
Umbel position	(i) axils of all leaves, and (ii) axils of upper leaves.
Flower size	mm; distance from calyx base to the corolla tip (10 categories).
Calyx size	mm; random undisturbed umbels, pedicel included (five categories).
Calyx teeth/calyx tube	(i) shorter, (ii) of the same length, and (iii) longer.
Calyx teeth form	(i) awl shaped, (ii) lanceolate, (iii) narrow triangular, and (iv) widely triangular.
Calyx indumentum	(i) glabrous, (ii) ciliate, and (iii) hairy.
Corolla color	(i) pale yellow, (ii) bright yellow, and (iii) dark yellow.

† After Chrtková-Zertková, 1973.

of the gels with stained bands were scanned with a color scanner (Epson ES-1200C, Epson America, Torrance, CA), and the image analyzed with NIH Image (National Institute of Health, Bethesda, MD) and Metaflo (The Valis Group, Richmond, CA) software using a 7200 Power Macintosh computer (Apple Computer, Cupertino, CA). Six primers known from prior experiments to be polymorphic among birdsfoot trefoil genotypes were used. The primers OPA-8, OPA-10, OPB-6, OPB-7, OPB-8, and OPB-13, met a selection criteria and produced 31, 27, 21, 18, 15, and 19 polymorphisms (131 bands total), respectively. The RAPD products were selected if (i) an intense band occurred in at least one accession, (ii) the band did not occur in all accessions, and (iii) the band was visualized in both replicates. The size of the RAPD product bands was determined by comparing their mobility to those of known standards using third-order polynomial regression equations.

Genetic distance ($d_{genetic}$) was estimated using:

$$d_{genetic} = 1 - a\ b^{-1} \quad [3]$$

where *a* is the number of shared RAPD product bands (bands present or absent), and *b* is the total number of bands scored.

Statistical Analyses

Symmetric Euclidean distance matrices were calculated from

$$A = \begin{bmatrix} a_{011} & a_{012} & \dots & a_{01j} \\ a_{021} & a_{022} & \dots & a_{02j} \\ \vdots & \vdots & \vdots & \vdots \\ a_{281} & a_{282} & \dots & a_{28j} \end{bmatrix}$$

where A is any $i \times j$ matrix with i equaling 1 to 28 of the trefoil genotypes and j equaling the number of levels measured for the descriptive variable(s). The Euclidean distance matrices were constructed using Systat 5.2.1 for the Macintosh (Evanston, IL). All levels of a descriptive variable were entered into a single line for each of the 28 genotypes and transposed, and then the STATS / CORR / EUCLIDIAN function was used to produce the distance matrix (D):

$$D = \begin{bmatrix} e_{0101} & e_{0102} & \dots & e_{0128} \\ & e_{0202} & \dots & e_{0228} \\ & & \dots & e_{ij} \\ & & & e_{2828} \end{bmatrix}$$

where e_{ij} off of the diagonal is the Euclidean distance for each of the 28 genotypes with the other 27 genotypes. The congruence of the geographic, ecologic, morphologic, and genetic classifications was determined by the product moment correlations (r) derived from the normalized Mantel Z (Mantel, 1967) using the MXCOMP command of NTSYSpc program, version 2.2 (Rohlf, 1997). In performing this test, if both matrices being compared contained corresponding distance estimates, then the value of the Z test criterion was large compared with chance expectation and was determined by the following equation:

$$Z = \sum_{i<j}^{n} x_{ij} y_{ij} \quad [4]$$

where Xij and Yij are two different measures relating to the *i*th element of a sample to the *j*th element in both matrices. This test required the calculation of symmetric distance matrices for each combination of classification characters. The estimated Z value was compared with its permutational distribution obtained from 500 random samples of all possible permutations of the matrices. The output of this test provides an empirical probability of obtaining a random Z value in excess of the estimated Z (Smouse et al., 1986).

The relationships of individual ecogeographic features to the genetic, morphologic, and geographic distances among the genotypes were also measured by the product moment correlation. The relationships of the quantitative morphologic characters with individual ecogeographic variables were tested using Pearson's r correlation coefficient (Snedecor and Cochran, 1980). Stepwise multiple correlation (Pearson's R collection coefficient) was used to determine which multiple combinations of ecogeographic variables were associated with morphologic traits. The relationships of individual qualitative morphologic traits with individual ecologic characteristics were determined using character states for a qualitative trait as the independent variable, and the quantitative value of the ecologic variable from each genotype collecting site as the dependent variable in a one-way analysis of variance using the F-statistic criterion (Snedecor and Cochran, 1980) for significance (Systat 5.2.1 for the Macintosh, Evanston, IL).

The placement of genotypes into the four RAPD cluster groups was validated using the RAPD cluster number as the classification factor in a stepwise discriminant analysis (SPSS 6.1 for the Macintosh, Chicago, IL). The test for significance of each RAPD product band was done using the univariate F-ratio (Hair et al., 1997). Based on the resulting analysis, 19 of 131 RAPD band products were identified as significantly contributing to the classification (OPA-8, 293 bp, 1205 bp, 1263 bp; OPA-10, 971 bp, 1009 bp, 1110 bp; OPB-6, 726 bp, 764 bp, 834 bp, 938 bp, 1158 bp, 1423 bp; OPB-7, 783 bp, 836 bp; OPB-8, 595 bp, 627 bp, 830 bp; and OPB-13, 1125 bp, 1542 bp) and retrospectively placed 100% of the genotypes correctly.

RESULTS AND DISCUSSION

Intra-Accession Diversity

The intra-accession genotype variation based on RAPDs was less than the variation among accessions ($P \leq 0.001$), indicating that a single genotype adequately represented each accession (Fig. 1). The bulked plant samples from all genotypes examined within an accession were also placed among the individual genotypes representing that accession. The RAPD results were supported by the morphologic examination, which also showed less variation among genotypes within an accession than among accessions (data not shown). Since birdsfoot trefoil is a highly variable species (Steiner, 1999a) and the accessions were chosen based on their anticipated diversity, it should be expected that variation among genotypes within an accession was less than the variation among accessions, even though this is a strongly cross-pollinated species. To identify unique birdsfoot trefoil accessions, a single genotype may suffice when screening large numbers of accessions.

Morphologic Classification

The 28 genotypes displayed polymorphism for both qualitative (Table 4) and quantitative (Table 5) morphologic characteristics within the range of traits reported by Chrtková-Zertová (1973). The genotypes were classified into five morphologic cluster groups (Fig. 2). The general appearance of the plants ranged from glabrous to pubescent leaves and erect to decumbent growth

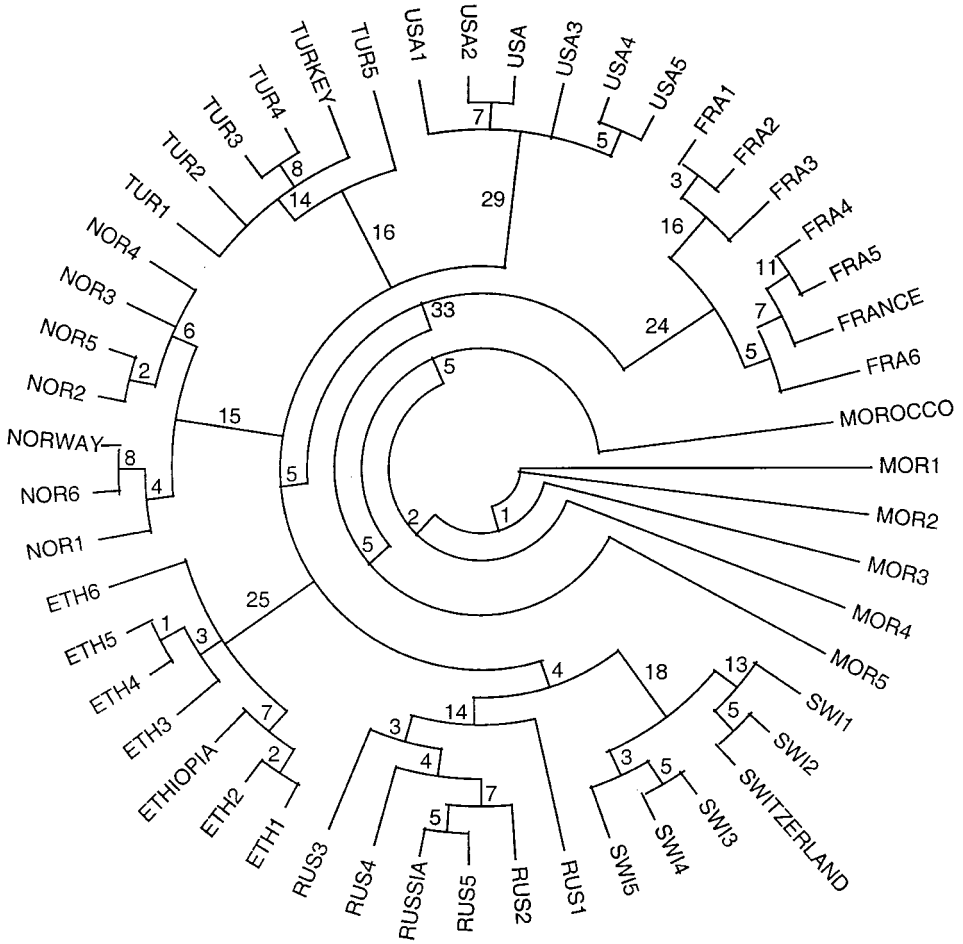

Fig. 1. Maximum parsimony analysis using a general heuristic search model using 130 polymorphic random amplified polymorphic DNA bands to assess the intra-accession genetic variation of five to six genotypes from seven National Plant Germplasm System exotic accessions and one domestic cultivar of birdsfoot trefoil. The birdsfoot trefoil name labels represent ETH, PI 26026; FRA, PI 235525; MOR, PI 31276; NOR, PI 319823; RUS, PI 325369; TUR, PI 464682; SWI, PI 234811; and USA, NC-83 (commercial cultivar); the accession names followed by a number indicate the genotype number examined within the accession. The full names of countries indicate the bulk samples of the approximate 15 genotypes from each accession. The numbers along branches indicate branch length.

habit. The genotypes mostly had solid stems, except for POL, RUS-1, SPA, and RUS-3, which had hollow stems. Leaf shape was variable, ranging from narrow, to obovate, to round leaf forms. The genotypes were also variable for leaf length and width with ETH, KAZ, RUS-4, and GEO-1 having the largest leaves. Both MOR and FRA-1 genotypes were rhizomatous. The rhizomes of FRA-1 and MOR differed in morphology, with MOR being more prolific. The FRA-1 genotype may be *L. corniculatus* var. *arvensis*, a naturally occurring progenitor of the cultivar Kalo, which is reported to be rhizomatous. The MOR genotype was from a recently discovered family of accessions collected in the Atlas Mountains of Morocco that were described as having rhizomes (Li and Beuselinck, 1996). Rhizomes in birdsfoot trefoil have also been described for birdsfoot trefoil L. *corniculatus* var. *crassifolius*, L. *corniculatus* var. *norvegicus*, and L. *corniculatus* var. *carnosus* (Chrtková-Zertová, 1973).

Most umbels were attached to the axilla of the upper leaves, except for the FRA-2, ETH, ITA-1, ITA-2, GRE-1, and GRE-2 genotypes, which had the umbels located in all leaf axilla. The genotypes were also variable for calyx traits, including teeth form and indumentum. The corolla color of most mature flowers was bright or dark yellow. Corolla color at the withering flower stage of development was either orange yellow or orange pink. The number of florets per umbel ranged from 1.6 (ETH) to 5.8 (RUS-3). The length of the calyx ranged from 5.6 to 9.8 mm for MOR and ETH, respectively. Total flower length ranged from 10.0 to 15.5 mm for UKR and GEO-1, respectively. The ETH genotype was autogamous, as all NPGS Ethiopian birdsfoot trefoil accessions generally are (Steiner and Poklemba, 1994). All other genotypes required hand manipulations to produce self-pollinated pods, if any (Garcia de los Santos et al., 2001).

Using the product moment correlation, morphologic similarities among genotypes were related to the closeness of the geographic proximity of their collecting sites (Table 6). The closer the genotype collecting sites were to one another, the more similar the genotypes were morphologically ($Z = 2.9$; $P \leq 0.001$). In the same way, the more similar the ecologic characteristics of the col-

Table 4. Morphologic descriptions of 28 exotic birdsfoot trefoil genotypes using 12 qualitative characters.†

	Whole plant				Leaflet			Calyx				
Identification	Growth habit	Underground shoots	Color	Stem Firmness	Form	Thick.	Indumentum	Influorescence position	Tube/ teeth ratio	Teeth form	Indumentum	Corolla color
MOR	D	P	G	S	OBC	SF	C	AUL	SL	L	C	PY
CZE	E	NP	BG	S	OBL	T	G	AUL	SL	NT	G	BY
IRA-1	A	NP	G	S	NO	T	C	AUL	SL	AS	C	PY
FRA-1	E	P	DG	S	OBL	SF	C	AUL	S	NT	H	BY
SWI	A	NP	DG	S	R	T	G	AUL	SL	AS	G	DY
FRA-2	A	NP	BG	S	R	F	C	AAL	L	AS	G	PY
MAC	D	NP	BG	S	NO	T	C	AUL	L	NT	H	BY
ETH	A	NP	BG	S	OBL	F	G	AAL	L	NT	C	DY
ITA-1	D	NP	BG	S	OBL	T	C	AAL	L	NT	C	BY
POL	E	NP	BG	H	OBL	T	G	AUL	S	NT	G	BY
UK	E	NP	DG	S	NO	T	G	AUL	L	NT	H	PY
GEO-2	A	NP	BG	S	R	T	H	AUL	L	NT	H	BY
KAZ	E	NP	BG	S	OBL	T	C	AUL	S	NT	C	BY
RUS-1	E	NP	DG	H	NO	T	G	AUL	SL	NT	C	PY
SPA	D	NP	DG	H	OBL	T	H	AUL	L	NT	H	DY
NOR-1	D	NP	BG	S	OBL	T	C	AUL	SL	NT	C	BY
NOR-2	D	NP	DG	S	OBO	T	G	AUL	S	NT	C	BY
RUS-2	A	NP	BG	S	OBL	SF	C	AUL	L	NT	H	DY
URK	E	NP	BG	S	R	T	C	AUL	SL	WT	H	BY
RUS-3	E	NP	BG	H	OBC	T	C	AUL	SL	NT	C	BY
TUR	D	NP	BG	S	R	T	C	AUL	SL	NT	C	BY
IRA-2	A	NP	DG	S	NO	T	C	AUL	S	WT	C	DY
GRE-1	A	NP	DG	S	OBL	T	C	AAL	S	L	C	PY
GRE-2	A	NP	DG	S	OBL	T	C	AAL	SL	L	H	BY
RUS-4	E	NP	BG	H	R	T	C	AUL	SL	NT	H	DY
GEO-1	E	NP	BG	S	R	T	C	AUL	SL	NT	C	BY
ITA-2	A	NP	DG	S	OBL	T	C	AAL	L	NT	C	BY
ROM	A	NP	DG	S	OBL	T	C	AUL	SL	NT	H	BY

† Growth habit: E = Erect, A = ascendent, D = decumbent; Underground shoots (Underg. shoot): P = present, NP = not present; Color of plant: G = glaucoish, BG = bright green, DG = dark green; Stem firmness (Stem firmn.): S = solid, H = hollow; Leaflets form: NO = narrow oblanceolate, OBL = oblanceolate, OBO = obovate, R = round, OBC = obcordate; Leaflet thickness (thick.): T = thin, SF = slightly fleshy, F = fleshy; Leaflet indumentum (indum.): G = glabrous, C = ciliate, H = hairy; Influorescence position (Infl. position): AAL = axilla of all leaves, AUL = axilla of upper leaves; Calix tube/calix teeth: S = shorter, SL = of the same length, L = larger; Calix teeth form: AS = awl shaped, L = lanceolate, NT = narrow triangular, WT = widely triangular; Calix indumentum: G = glabrous, C = ciliate, H = hairy; Color of corolla: PY = pale yellow, BY = bright yellow, DY = dark yellow.

lecting site were, the more similar was the genotype morphology. The geographic and ecologic classifications were highly similar ($Z = 3.7$; $P \leq 0.001$). The only ecologic descriptors that were not related to geographic distance were snow, high temperature, and precipitation (Table 7).

General morphologic-classification similarities were most significantly attributed to low temperature, amount

Table 5. Description of six quantitative morphologic characters for 28 exotic birdsfoot trefoil genotypes.

	Character					
Identification	Leaflet width	Leaflet length	Floret number	Peduncle length	Flower size	Calyx size
	mm				mm	
MOR	2.5 ± 0.3	6.4 ± 0.2	1.7 ± 0.1	30.3 ± 13	11.5 ± 0.2	5.6 ± 0.1
CZE	3.7 ± 0.6	8.4 ± 0.4	4.6 ± 0.3	24.2 ± 12	13.2 ± 0.2	6.8 ± 0.1
IRA-1	2.5 ± 0.2	7.6 ± 0.1	1.8 ± 0.2	48.5 ± 10	10.6 ± 0.2	5.7 ± 0.2
FRA-1	3.5 ± 0.4	5.8 ± 0.1	3.6 ± 0.2	52.8 ± 11	13.6 ± 0.2	6.1 ± 0.2
SWI	3.8 ± 0.3	7.2 ± 0.1	2.4 ± 0.2	33.8 ± 8	13.8 ± 0.2	6.4 ± 0.1
FRA-2	3.7 ± 0.4	9.1 ± 0.3	2.0 ± 0.2	36.6 ± 5	12.3 ± 0.1	8.4 ± 0.3
MAC	3.4 ± 0.2	7.3 ± 0.2	3.9 ± 0.3	48.2 ± 7	13.0 ± 0.2	9.2 ± 0.1
ETH	5.2 ± 0.3	10.2 ± 0.3	1.6 ± 0.1	32.7 ± 8	11.4 ± 0.2	9.8 ± 0.1
ITA-1	2.8 ± 0.2	7.0 ± 0.2	4.1 ± 0.1	77.8 ± 9	14.8 ± 0.1	7.8 ± 0.1
POL	4.1 ± 0.6	8.7 ± 0.3	4.7 ± 0.2	46.5 ± 13	11.6 ± 0.1	6.5 ± 0.1
UK	4.0 ± 0.6	8.1 ± 0.6	3.4 ± 0.2	49.3 ± 6	12.8 ± 0.2	7.6 ± 0.1
GEO-2	4.2 ± 0.5	7.8 ± 0.2	5.0 ± 0.2	72.1 ± 7	15.0 ± 0.1	8.8 ± 0.2
KAZ	7.1 ± 0.7	12.1 ± 0.3	4.3 ± 0.1	81.8 ± 15	14.0 ± 0.2	7.9 ± 0.2
RUS-1	3.9 ± 0.4	9.0 ± 0.2	4.7 ± 0.4	33.8 ± 7	13.0 ± 0.2	8.5 ± 0.1
SPA	2.4 ± 0.3	5.9 ± 0.1	3.2 ± 0.1	40.6 ± 7	12.2 ± 0.1	7.8 ± 0.2
NOR-1	3.9 ± 0.4	8.8 ± 0.2	5.5 ± 0.2	52.3 ± 7	14.5 ± 0.1	7.9 ± 0.2
NOR-2	3.6 ± 0.4	8.6 ± 0.2	4.8 ± 0.2	63.6 ± 7	15.0 ± 0.1	7.6 ± 0.2
RUS-2	4.1 ± 0.6	9.0 ± 0.2	3.6 ± 0.3	63.4 ± 6	12.6 ± 0.3	7.3 ± 0.1
UKR	4.2 ± 0.8	7.6 ± 0.2	2.8 ± 0.3	91.1 ± 14	10.0 ± 1.5	6.7 ± 0.2
RUS-3	3.5 ± 0.6	8.3 ± 0.4	5.8 ± 0.3	51.5 ± 7	13.8 ± 0.2	7.1 ± 0.2
TUR	4.6 ± 0.6	7.3 ± 0.3	3.9 ± 0.2	46.1 ± 8	10.9 ± 0.2	6.1 ± 0.1
IRA-2	31. ± 0.6	7.9 ± 0.1	2.1 ± 0.1	42.2 ± 6	12.9 ± 0.3	7.0 ± 0.1
GRE-1	3.1 ± 0.6	8.8 ± 0.4	3.1 ± 0.2	51.9 ± 5	14.5 ± 0.3	6.9 ± 0.2
GRE-2	3.7 ± 0.4	8.8 ± 0.3	3.3 ± 0.2	46.6 ± 6	14.8 ± 0.1	9.2 ± 0.1
RUS-4	4.5 ± 0.6	12.2 ± 0.3	5.4 ± 0.2	52.2 ± 4	14.8 ± 0.1	7.8 ± 0.1
GEO-1	5.1 ± 0.8	9.6 ± 0.1	4.1 ± 0.2	63.7 ± 5	15.5 ± 0.1	8.2 ± 0.2
ITA-2	3.4 ± 0.3	7.4 ± 0.2	3.1 ± 0.2	58.3 ± 3	11.9 ± 1.6	9.5 ± 0.1
ROM	2.7 ± 0.3	7.0 ± 0.1	4.3 ± 0.2	53.9 ± 4	14.2 ± 0.1	8.6 ± 0.1

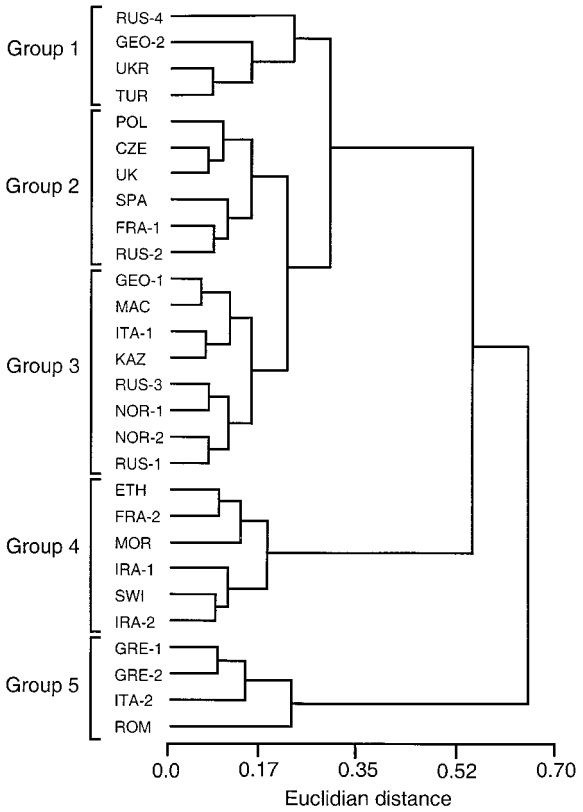

Fig. 2. Ward's cluster analysis classification of 28 exotic Old-World birdsfoot trefoil genotypes based on 18 morphologic traits described by 71 binary character states using Euclidean distance. Genotype name labels are defined in Table 1.

of sunshine, elevation, and latitude (Table 7). Among the quantitative morphologic traits, the expression of number of florets per umbel was greatly affected by ecogeographic factors, while leaf dimensions and calyx length were mostly unaffected (Table 8). There was a great deal of collinearity among many of the ecologic characteristics (19 of 37 possible correlation comparisons were associated at $P \leq 0.05$). Using stepwise multiple correlation, the number of florets per umbel was most significantly influenced by the amount of sunshine and low temperature of the collecting sites ($R = 0.86$; $P \leq 0.0001$). Sunshine and low temperature were collinear ($r = 0.53$; $P \leq 0.004$). The only other quantitative

Table 6. Product moment correlations (r) from the normalized Mantel Z statistic for comparisons of different proximity matrices using random amplified polymorphic DNA (RAPD) bands, and combinations of plant morphologic and collecting site ecologic characteristics and geographic distances from 28 exotic birdsfoot trefoil genotypes.

Descriptor	Genetic†	Geographic‡	Ecologic§
		r-value	
Morphologic#	0.15	0.25*	0.34**
Genetic	–	0.10	0.21*
Geographic		–	0.39**

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
† Genetic distances calculated in PAUP using 131 RAPD bands.
‡ Geographic distances.
§ Ecologic distances calculated using six descriptors that included: lowest and highest monthly temperature, annual average temperature, monthly and annual accumulated precipitation (precipitation), monthly and annual accumulated snow depth (snow), monthly and annual percentage of sunshine hours (sunshine).
# Morphologic distances calculated using 18 morphologic traits using 71 binary characters states using PAUP.

morphologic trait correlated with an ecogeographic variable was leaf length with longitude. Leaf length increased as the genotype collecting-site location moved east (Table 8). The ecogeographic data may have limited accuracy because they were obtained from global-level data sets (Estes and Mooneyhan, 1994; Steiner and Greene, 1996). However, the 6500-km maximum geographic distance and associated ecogeographic differences between collecting sites likely had a greater influence on genotype differences than inaccuracies in the global-level data sets.

Genotypes collected at lower latitudes tended to display their umbels in the upper leaf axilla more than those collected at higher latitudes, which had the umbels distributed along the length of the stems (37° vs. 46° N, respectively; Table 9). Genotypes that bore umbels in the upper leaf axilla were found more in warmer than cooler environments (14.9°C vs. 6.2°C, respectively). This may be an adaptive feature for cooler climates where the number of growing degree days are limited, but day length is long during the reproductive period. Such a mechanism could maximize the number of seeds that are produced by producing inflorescences at all stem nodes, rather than only at the terminal end of a stem.

Plants with decumbent growth tended to be located

Table 7. Product moment correlations (r) from the normalized Mantel Z statistics for association among genetic, morphologic, and geographic classifications with nine ecogeographic characteristics from the collecting sites of 28 exotic birdsfoot trefoil genotypes.

	Ecogeographic characters†								
		Temperature					Geography		
Classification	Snow	Average	Low	High	Sunshine	Precipitation	Elevation	Latitude	Longitude
	r-value								
Genetic‡	−0.19	0.16	0.20*	0.10	0.22*	0.06	0.09	0.09	0.00
Morphologic§	0.00	0.24*	0.24*	0.13	0.48**	0.09	0.20*	0.34*	0.04
Geographic¶	0.16	0.25**	0.40**	0.00	0.45**	0.08	0.35**	0.58**	0.73***

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Matrices constructed from single ecogeographic characters as given in tables 1 and 2.
‡ Matrix of distances constructed from 130 random amplified polymorphic DNA bands using PAUP.
§ Matrix calculated using 18 morphologic characters in 71 binary character states.
¶ Matrices constructed from geographic distances.

Table 8. Pearson's correlation coefficients (*r*) between four quantitative morphologic and nine ecogeographic characters from 28 exotic birdsfoot trefoil genotypes.

	Morphologic descriptors			
Descriptor	Leaf length	Leaf width	Calyx length	Floret number
	r			
Snow	0.28	0.36	0.04	0.62***
Average temperature	−0.11	−0.26	0.01	−0.71***
Low temperature	−0.29	−0.37	0.01	−0.74***
High temperature	0.10	−0.11	−0.04	−0.45**
Sunshine	−0.05	−0.05	−0.06	−0.76***
Precipitation	−0.20	−0.16	0.09	0.14
Elevation	−0.16	−0.04	−0.14	−0.44**
Latitude	0.11	−0.01	−0.10	0.75***
Longitude	0.42*	0.36	0.07	0.16

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

more at lower elevations (233 m) than plants with ascending (832 m) and erect (926 m) growth. Decumbent growth was more common at higher latitudes (50.2° N) than ascending plants (38.5° N). Glaucous-colored genotypes (MOR and IRA-1) were found only at high altitudes (1830 m). Also, light green and dark green plants were both found at low average altitudes except for SWI, ETH, and NOR-2. All of these findings generally agree with those of Chrtková-Zertová (1973).

Leaves with ciliate indumentum were found more in regions with lower precipitation and higher average temperature (523 mm and 20.9°C, respectively) than leaves with glabrous (924 mm and 15.2°C, respectively) and hairy (1302 mm and 12.5°C, respectively) indumentum. No differences were found between the ecologic variables and the plant classes described as glabrous- and hairy-leaf indumentum. This finding differs from the observation of Chrtková-Zertová (1973), as does the finding that more genotypes with thicker leaves were collected in warm and sunny environments at low latitudes than at cool and cloudy environments.

The finding of associations among birdsfoot trefoil morphologic traits and ecogeographic characteristics of the genotype collecting sites is supported by other research, including associations between altitude and morphologic traits (Small et al., 1984), agronomic forage quality factors with geographic origins (McGraw et al, 1989), seed proteins with accession distributions across broad geographic regions as well as with collecting-site elevations (Steiner and Poklemba, 1994). Re-analysis of the Chrtková-Zertová (1973) data showed a trend of widely distributed genotypes being found in warmer climates, and more geographically restricted genotype distributions being found in cooler regions along an environmental gradient from southern European montaine habitats to subarctic boreal regions at high latitudes (Steiner, 1999a). These findings indicate that specific morphologic traits are important for the natural adaptation of genotypes to specific habitats. The function and utility of these traits for developing improved birdsfoot trefoil cultivars for specific production environments still needs to be determined.

Genetic Classification

The genotype classification based on 130 polymorphic RAPD bands produced four genetic groups (Fig. 3). Several polymorphic bands were observed for each primer, and most primers produced band sizes ranging from 293 bp to 1880 bp. The four genetic groups based on 131 RAPD bands were used as classes in a stepwise multiple discriminant analysis to validate the cluster analysis genetic classification of the genotypes. Nineteen of the bands were identified as significant ($P \leq 0.05$) descriptors that differentiated the genotypes into the four groups and produced a canonical correlation value of 0.999. One hundred percent of the accessions were correctly placed by the stepwise discriminant analysis function.

Unlike the relationship between geographic distance and plant morphology, no relationship was found between genotypes for geographic and genetic distances (Table 6). The close proximity of collecting sites to one another did not ensure genetic similarity based on RAPD markers. However, RAPD genetic similarity among genotypes was associated with the ecologic similarity of the collecting sites. Thus, geographically sepa-

Table 9. Analysis of variance relationships for 10 qualitative morphologic characters with nine ecogeographic characters from 28 exotic birdsfoot trefoil genotypes.

	Qualitative morphological descriptors									
Descriptor	Umbel position	Calyx ratio	Leaf indumentum	Calyx teeth form	Corolla color	Leaf form	Plant color	Leaf thickness	Calyx indumentum	Growth habit
Snow	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Average temperature	****	ns	ns	ns	ns	ns	ns	*	ns	ns
Low temperature	***	ns	ns	ns	ns	ns	ns	**	ns	ns
High temperature	ns	ns	**	ns	ns	ns	ns	ns	ns	ns
Cloudiness	ns	ns	ns	**	ns	ns	*	*	ns	*
Precipitation	ns	ns	*	ns	ns	****	ns	ns	ns	ns
Elevation	ns	ns	ns	ns	ns	ns	*	ns	ns	*
Latitude	*	ns	ns	ns	ns	ns	ns	*	ns	*
Longitude	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Number of character states	2	3	3	4	3	5	4	3	3	3

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
**** Significant at the 0.0001 probability level.
ns = not significant

rate sites that are characterized by similar ecologic conditions may be more likely to have genotypes with similar genetic backgrounds, compared with collecting sites that are geographically close but differ ecologically. For example, RAPD Group-1 genotypes were collected over a large geographic range, but came from the lowest average latitudes (36.3° N) of all collecting sites and received the greatest amount of average annual sunshine (60%) of the four RAPD classification groups. These genotypes were mostly found near the Mediterranean Sea or in Asia Minor. RAPD Group-2 genotypes were collected at the greatest average latitude (50.1° N) and had the least amount of average sunshine (39%) of all classification groups (the greater the collecting-site latitude, the lower the sunshine percentage; $r = -0.79$; $P \leq 0.0001$). These collecting sites ranged widely across most of northern continental Europe, but have similar ecogeographic influences. On the other hand, genotypes in RAPD Group-3 from southeastern Europe and Asia Minor were primarily from a more limited geographic range than those from Group-1 and Group-2. RAPD Group-4 genotypes were the most genetically divergent from the genotype of the other three groups and from southeastern Europe.

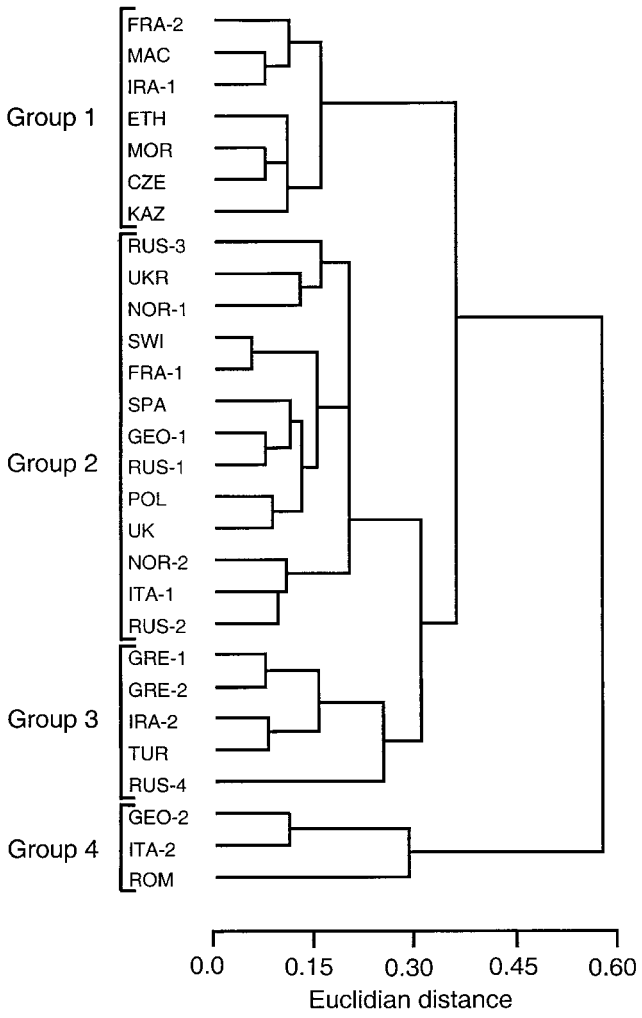

Fig. 3. Classification of 28 exotic Old-World birdsfoot trefoil genotypes based on 130 polymorphic random amplified polymorphic DNA band products using Euclidean distance and Ward's cluster technique. Genotype name labels are defined in Table 1.

The significant associations for collecting-site geographic closeness with genotype morphology, and the collecting-site ecology with plant morphology and genetic similarity suggest that either similar genotypes from a common progenitor migrated great distances to become established in habitats with similar climatic conditions, or that conserved traits with adaptive values to habitats were naturally selected from a highly diverse birdsfoot trefoil progenitor population as the species migrated across the landscape. Examples for both of these cases have been presented for other species (Sauer, 1988). The absence of a relationship between the morphology and genetic similarities suggests that specific traits with adaptive value may have accumulated in habitats subjected to similar ecologic conditions, with less regard for the genetic origins of the progenitors. The deviation between genetic and morphologic classifications may be due to the conservation of morphologic traits under natural selection in similar environments, in spite of the occurrence of random mutations that would tend to cause eventual differences among geographically distant populations (Johns et al., 1997). Additional research is needed to determine if the underlying genetic basis of traits adapted to specific environments was due to convergent phenotypes and could be tested by crossing morphologically similar, but genetically divergent, individuals. Populations exhibiting similar molecular composition but dissimilar morphology have resulted from recent selection pressures with few gene changes (Crawford, 1990). Single genes can have a significant effect on morphologic trait expression (Beer et al., 1993). This may be regardless of the nature or amount of genetic diversity that is present to be selected upon by the environment. It is possible that single-trait differences among genotypes that affect morphology may not be detected by RAPDs. However, this may not exclusively be so for birdsfoot trefoil because great genetic distances based on RAPDs have been shown for induced photoperiod mutants (Steiner and Beuselinck, 2001).

Since genotype genetic similarities were associated with the ecologic features of the collecting sites and not with plant morphology, the classification of birdsfoot trefoil germplasm should not rely solely on geographic distance or morphologic descriptions, but also consider ecogeographic and RAPD genotype diversity. Even though geographic distance among collecting sites was highly associated with ecologic distance (Table 6), geographic distance alone would not be adequate to classify germplasm collections because adaptive features or unique genetic combinations may not be recognized. For example, the genotypes NOR-1 and NOR-2 (which are more similar genetically than morphologically; Fig. 2 and 3) are from collecting sites geographically close to one another, but ecologically diverse due to difference in elevation, precipitation, and snowfall (Table 2). Genotypes IRA-1 and IRA-2 are from sites that are of

close geographic and ecologic proximity and are similar morphologically, but differ genetically.

Low temperature and sunshine were associated with the genetic classification of genotypes (Table 7), so these collecting-site ecologic characteristics may be important criteria when selecting birdsfoot trefoil germplasm. However, the scale of the geographic area encompassing a collection description may influence its ecologic interpretation (Steiner, 1999b), so the generality of this approach needs to be tested with other species and using different geographic scales.

These findings support the contention that specific traits may be associated with native-habitat conditions and thus provide insights into adaptive mechanisms that could be useful for crop improvement (Rick, 1973). These findings also strengthen the opinions that useful germplasm may be identified in regions that are ecologically distinct from those where germplasm has previously been obtained (Steiner and Poklemba, 1994; von Bothmer and Seberg, 1995).

CONCLUSIONS

The 28 birdsfoot trefoil genotypes examined represented a range of diversity across the Old-World and displayed polymorphism for both qualitative and quantitative morphologic characteristics. Morphologic similarities among the genotypes were related to the general geographic proximity of their collecting sites to one another, and with the general ecology of the collecting-site habitats. Specific morphologic traits were found to be related to specific ecogeographic features of the collecting-site habitats. Of agronomic importance, floral morphology was related to collecting-site latitude and average temperature, and may have adaptive significance for natural reseeding in habitats with short growing seasons. Plant growth form was correlated with collecting-site elevation and latitude.

Geographic distance among genotype collecting sites was not associated with plant genetic distance, but the ecologic similarity was related to genetic similarity. The genotypes were related to similar ecologic features among collecting sites found in broad geographic regions that included the Mediterranean Basin/Asia Minor, northern continental Europe, southeast Europe, and southern Europe. The similarity between the genetic and ecologic classifications suggests that germplasm adapted to similar habitats, even if geographically distant, have acquired similar phenotypes. The RAPD descriptors were associated with the ecologic similarity of genotype collecting sites but not with their geographic closeness, so classifications of germplasm should not only use germplasm ecogeographic or morphologic characteristics, but a combination of descriptor types. The utility of combining genetic (RAPD), morphologic, and ecologic characteristics reveals combinations of variation among the birdsfoot trefoil genotypes that would not be apparent with any single measurement, and could provide a more complete understanding of the diversity in birdsfoot trefoil and other species germplasm collections.

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